Non-medical prescription opioid users exhibit dysfunctional physiological stress responses to social rejection

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DOI: https://doi.org/10.1016/j.psyneuen.2018.09.023

tion opioid users exhibit dysfunctional physiological stress responses to social rejection. Psychoneuroendocrinology, 100:264-275.
DOI: https://doi.org/10.1016/j.psyneuen.2018.09.023

Accepted Manuscript

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PII: S0306-4530(18)30635-8
DOI: https://doi.org/10.1016/j.psyneuen.2018.09.023
Reference: PNEC 4073

To appear in:

Received date: 26-6-2018
Revised date: 7-9-2018
Accepted date: 16-9-2018

Please cite this article as: Kroll SL, Williams DP, Thoma M, Staib M, Binz TM, Baumgartner MR, Kirschbaum C, Thayer JF, Quednow BB, Non-medical prescription opioid users exhibit dysfunctional physiological stress responses to social rejection, *Psychoneuroendocrinology* (2018), https://doi.org/10.1016/j.psyneuen.2018.09.023

Non-medical prescription opioid users exhibit dysfunctional physiological stress responses to social rejection

Running title: Social rejection and opioid use

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Abstract: 248 words
Main text: 6552 words
Tables: 2
Figures: 4

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Highlights

- Non-medical prescription opioid use (NMPOU) has reached epidemic proportions
- We investigated stress responses to social rejection in persons with chronic NMPOU
- High opioid craving was associated with worse parasympathetic response to social stress
- NMPOU group displayed increased neuroendocrine stress response to social exclusion

- Physiological stress responses are likely dysregulated by chronic NMPOU

Abstract

Non-medical prescription opioid use (NMPOU) recently increased dramatically, especially in the U.S. Although chronic opioid use is commonly accompanied by deficits in social functioning and dysregulation of the hypothalamic-pituitary adrenergic (HPA) stress axis, little is known about the impact of NMPOU on psychosocial stress responses. Therefore, we measured physiological responses of the autonomic nervous system and the HPA axis to social rejection using the Cyberball paradigm. We compared 23 individuals with NMPOU, objectively confirmed by hair and urine analyses, with 29 opioid-naïve, healthy controls. As expected, heart rate variability (HRV), an index of parasympathetic activity, increased significantly during exclusion within controls, while in the NMPOU group only a trend in the same direction was found. However, increased HRV was robustly moderated by opioid craving indicating worse emotion regulation to social exclusion specifically in individuals with high opioid craving. Greater levels of the adrenocorticotropic hormone and cortisol responses to social rejection were found in the NMPOU group indicating hyperreactivity of the HPA axis to social exclusion. Self-ratings suggest that opioid users were aware of rejection, but less emotionally affected by exclusion. Furthermore, controls showed greater negative mood after the Cyberball confirming the task's validity. Moreover, NMPOU individuals reported a smaller social network size compared to controls. Present findings suggest that chronic NMPOU is associated with dysfunctional physiological responses to psychosocial stressors such as social rejection. In sum, NMPOU was associated with poorer regulation of the parasympathetic nervous system, especially under opioid craving highlighting its potential importance in relapse prevention.

Keywords: morphine; opioids; social pain; ostracism; ACTH; codeine

1. INTRODUCTION

In recent decades, the misuse of prescription opioids (e.g., morphine, fentanyl, oxycodone, or codeine) has increased dramatically. Especially in the U.S., non-medical prescription opioid use (NMPOU) has reached epidemic dimensions, with a past-year prevalence of 37.8%, of which 12.5% fulfilled opioid misuse criteria (Han et al., 2017). Accordingly, NMPOU-related deaths dramatically increased by 265% from 2012 to 2015 (UNODC, 2017). Importantly, a relapse rate of up to 91% after detoxification for opioids is higher than for any other drug of abuse (Smyth et al., 2010), thereby signifying a lack of effective long-term treatments for opioid abstinence. A crucial factor for preventing relapse of drug addiction is functional social support (Ellis et al., 2004; Havassy et al., 1991), while opioid use is commonly accompanied by deficits of social functioning (Darke, 2011). For example, we recently found inferior performance in understanding and recognizing others' feelings and emotions in individuals with NMPOU compared to healthy controls, which is crucial for prosocial behavior and interpersonal relationships (Kroll et al., resubmitted). Furthermore, the Brain Opioid Theory of Social Attachment (BOTSA), initially formulated by Panksepp et al. (1978), proposed an association between the opioid system and social bonding, including social rejection. In line with the BOTSA, μ-opioid receptor (MOR) agonists might induce feelings of social comfort and subsequently reduce affiliative behavior. Findings in animal studies supported this assumption, reporting relief from separation distress measured by reduced isolation calls and decreased affiliative behavior such as social grooming after administration of MOR agonists (Machin and Dunbar, 2011). Accordingly, MOR antagonism increased separation distress and subsequently motivation to seek social contact in animals to counteract this negative state. In line with these findings, a recent study in humans reported reduced feelings of connection with close others in the laboratory and also in day-to-day reports after administration of the MOR antagonist naltrexone (Inagaki et al., 2016). Furthermore, decreased activity of the endogenous μ-opioid system, as measured by an individual's pain tolerance, was linked to smaller social network size (Johnson and Dunbar, 2016). Additionally, low baseline MOR availability measured using positron emission tomography (PET) was associated with avoidant attachment style, further supporting the BOTSA in humans (Nummenmaa et al., 2015). Additional neuroimaging studies using PET and functional magnetic resonance imaging (fMRI) reported

increased neuronal and MOR activity during social rejection, specifically in the dorsal anterior cingulate cortex (dACC), amygdala, anterior insula, and prefrontal cortex (PFC) (Cacioppo et al., 2013; Hsu et al., 2013). Interestingly, these brain areas are also associated with high MOR density and the affective pain system indicating a neuronal overlap of representations of physical and social pain, i.e., social rejection, exclusion, or ostracism (Eisenberger, 2015; Woo et al., 2014).

Social pain is commonly measured by the Cyberball paradigm in humans, a virtual ball-tossing game, where participants were asked to play a computer game with two other players, who are in reality controlled by the experimenter to ostensibly exclude or include the participant (Williams and Jarvis, 2006). Aside from neurocortical activation during social rejection, social pain is also linked to the social stress response of the autonomic nervous system (ANS) and the hypothalamic-pituitary adrenergic (HPA) axis. ANS response to social rejection has been reported in healthy subjects, as indexed by heart rate deceleration and altered skin conductance level (SCL) (Kelly et al., 2012; Moor et al., 2010; van der Veen et al., 2014). Furthermore, increased cortisol levels have been found after social exclusion, indicating activation of the HPA axis (Blackhart et al., 2007; Zwolinski, 2008). However, evidence of the ANS and HPA axis stress response to social pain is inconsistent, with other studies being either unable to replicate the reported findings or, rather, providing contrary results (Bass et al., 2014; Jobst et al., 2015; Radke et al., 2018; Zwolinski, 2012). Two recent studies from the same group investigated acute effects of the partial MOR agonist buprenorphine on psychosocial stress. They reported decreased feelings of social rejection using the Cyberball task and attenuated cortisol levels after psychosocial stress induced by the Trier Social Stress Test (TSST) in healthy buprenorphine-administered participants (Bershad et al., 2015; Bershad et al., 2016). Thus far only one study has investigated the impact of NMPOU on psychosocial stress processing: Back et al. (2015) reported no differences in heart rate (HR) and saliva cortisol levels between healthy controls and individuals with prescription opioid dependence after the TSST. However, in this study cortisol levels were measured in the morning, which is a suboptimal time to assess the stress-related cortisol response, due to its diurnal rhythm and ceiling effects (Kirschbaum et al., 1999).

The ANS dually innervates peripheral organs including the heart. In a resting state, ANS influence is characterized by a relative dominance of the parasympathetic nervous system (PNS) over influences of the sympathetic nervous system (SNS) (Thayer and Lane, 2009). PNS control adaptively regulates physiological functions to produce context appropriate responses via the vagus nerve (i.e., immune, inflammatory, and cardiac functions) (Thayer and Sternberg, 2006; Weber et al., 2010). Converging evidence suggests that heart rate variability (HRV), defined as the rapid beat-to-beat fluctuations in a HR time series, serves as an index of PNS activity (Thayer et al., 2012). Importantly, a recent meta-analysis showed a significant relationship between resting HRV and regional cerebral blood flow in the ventromedial PFC, in addition to the left amygdala (Thayer et al., 2012). Brain areas involved in both processing and regulating emotions include the amygdala (up-regulation of negative emotions) and frontal brain regions such as the PFC (down-regulation of negative emotions) (Etkin et al., 2011). Therefore, HRV is widely considered as a physiological index of emotion regulation (Thayer and Lane, 2000; Williams et al., 2015). Importantly, studies have yet to investigate how HRV responses to negative emotions may differ between healthy controls and individuals in a NMPOU group.

Although reported findings in animals and humans indicate that the μ-opioid system might play a crucial role in interpersonal behavior and processing of social rejection, studies investigating social pain in individuals with chronic NMPOU are still lacking. Therefore, the aim of our study was to assess stress response to social rejection on a subjective and physiological level in individuals with NMPOU compared to healthy matched controls. We collected neuroendocrine and electrophysiological data in order to measure the activity of the ANS, PNS, and HPA axis during the Cyberball paradigm. Furthermore, participants were asked for their subjective evaluation of the social exclusion and their mood before and after the Cyberball Game. Additionally, social network size of the participants was assessed by a questionnaire. Based on reported dampening effects of acute opioid administration on social stress (Bershad et al., 2015; Bershad et al., 2016), we hypothesized that individuals with NMPOU would be less affected by social rejection compared to controls, reflected in reduced subjective and physiological stress responses. In accordance with the

BOTSA, we further expected that individuals with NMPOU would reveal a smaller social network size than controls, due to decreased affiliative behavior.

2. METHODS

2.1. Participants

The sample consisted of 23 individuals with NMPOU and 29 opioid-naïve healthy controls matched for sex, age, years of education, and smoking status. Participants were recruited through advertisements in internet forums, local newspapers, and through specialized addiction centers. General exclusion criteria were neurological disorders or head injuries, severe physical diseases (e.g., HIV, HCV, or diabetes), axis-I DSM-IV and DSM 5 psychiatric disorders (except for alcohol and nicotine use disorders as well as former depressive episodes), chronic pain disorder, recent emotional painful events (e.g., breakup of a relationship or death of a close friend/relative), and insufficient proficiency in German language. Substance use disorder was not an exclusion criterion for the NMPOU group, although participants showing any intravenous (i.v.) drug use or history of street heroin dependence were excluded. The inclusion criterion for opioid users was NMPOU over at least the last six months. Participants were instructed to abstain from psychotropic substances for 72h and for 24h from alcohol. Furthermore, opioid users were asked to abstain from opioids on the testing day, or to take an adequate and minimized dose of opioids, if necessary, which solely removed withdrawal symptoms, to avoid measuring acute or withdrawal effects. Opioid use was objectively determined by urine and hair toxicology analyses using a semi-quantitative enzyme multiplied immunoassay method and liquid chromatography-tandem mass spectrometry, respectively (for technical details see Kroll et al., resubmitted). The study was approved by the Cantonal Ethics Committee of Zurich. All participants provided written informed consent and received compensation for their participation.

2.2. Procedure

The Structured Clinical Interview for axis-I DSM-IV Disorders (SCID-I), adapted for DSM-5 regarding substance use disorders, was carried out by trained psychologists at the beginning of the testing day (Wittchen et al., 1997). Substance use was assessed by means of a standardized and structured Interview for Psychotropic Drug Consumption (Quednow et al., 2004). Current opioid craving was assessed by a Numeric Rating Scale (NRS) from one (no craving) to ten (highest craving) and by the Objective Opioid Withdrawal Scale (OOWS) (Handelsman et al., 1987). Additionally, self-report questionnaires were applied to determine severity of nicotine dependence (Fagerström Test of Nicotine Dependence, FTND) (Heatherton et al., 1991) and depressive symptoms (Beck Depression Inventory, BDI) (Beck et al., 1961). Premorbid verbal IQ was assessed with a German vocabulary test – the Mehrfachwahl-Wortschatz-Intelligenztest (MWT-B) (Lehrl, 1999). After clinical assessments, an i.v. catheter was placed in the forearm vein of the non-dominant hand by a trained and certified psychologist approximately one hour prior to the first blood sample. Following the Cyberball paradigm, a neuropsychological test battery was applied, the results of which have been outlined elsewhere (Kroll et al., resubmitted).

2.3. Cyberball paradigm

All participants were seated in a soundproof experimental room at a distance of 60 cm to the screen, where the Cyberball game was played (Williams and Jarvis, 2006). Participants were told that the task assessed performance of mental imagery and that therefore they should try to imagine the ball-tossing situation as realistically as possible. To increase the credibility of the game, we implemented a photo of each participant and of the two teammates, who were in reality members of our group, within the game and introduced them personally prior to the Cyberball. After the task started, participants were included in the game for about one minute and received the ball six times (10%). Subsequently, participants were excluded and did not receive the ball anymore for the next two minutes, as controlled by the computer. The total duration of the game was about three minutes (60 throws). To assess mood responses to social rejection, the Positive and Negative Affect Schedule (PANAS) (Watson et al., 1988) was used before and after the Cyberball. Differences in positive (PA)

and negative affect (NA) were measured on a five-point Likert scale. Furthermore, participants completed a questionnaire after the Cyberball where they were asked how excluded and included they felt on a nine-point Likert scale, how often they received the ball in percentage, and how difficult it was to believe that they were playing the ball-tossing game with real persons without seeing them (1= “not at all” to 5= “very difficult”).

2.4 Social Network Questionnaire (SNQ)

Based on the social contact circle interview (Linden et al., 2007), the SNQ evaluates the amount of social contacts in specific areas of life such as household, family, work or apprenticeship, friends, neighbors, (sport) clubs or unions, and others. Only direct contact over the last four weeks such as via personal encounters, telephone, email, or letter was included. For calculating the total social network size, participants were told to name contacts only once within the SNQ to avoid double entries of contacts in different areas.

2.5. Physiological stress responses to social rejection

2.5.1. Electrophysiological responses

Spontaneous fluctuation (SF) in skin conductance during the Cyberball was assessed as an index for sympathetic activity (Bach et al., 2011). Skin conductance was recorded on the thenar/hypothenar of the non-dominant hand using two 8mm disc Ag/AgCl cup electrodes (EL258, Biopac Systems Inc., Goleta, CA) and 0.5% NaCl gel (GEL101, Biopac Systems Inc.) (Hygge and Hugdahl, 1985). Skin conductance signal was amplified with a skin conductance response (SCR) coupler/amplifier (V71-23, Coulbourn Instruments), digitized at 1000 Hz using a Dataq card (DI-149, Dataq Inc., Acron, OH), and recorded with Windaq (Dataq Inc.) software. SF over the total Cyberball game and during exclusion (60 sec after Cyberball onset) was analyzed with the Matlab toolbox for psychophysiological modeling, PsPM 4.0 (pspm.sourceforge.net) using a dynamic causal model (DCM). The DCM computes a non-linear estimation of the number of SF with a detection threshold of 0.1 μS and provides the most sensitive indicator for SF (Bach et al., 2011). Additionally, we report sympathetic

activity based on classical skin conductance level (SCL) representing the mean signal over all epochs in μS.

During the Cyberball game, cardiac activity was recorded via a three-lead electrocardiogram (ECG) at 1000 Hz sampling rate with 45-mm, pregelled Ag/AgCl adhesive electrodes attached below the right clavicle, on the left side of the abdomen below the heart, and on the right side of the abdomen, as used in prior studies (Williams et al., 2015; 2017). Data were preamplified and 50 Hz notch-filtered with a Coulbourn isolated five-lead amplifier (LabLinc V75-11, Coulbourn Instruments) and recoded with Windaq (Dataq Inc.) software. ECG data were analyzed using Kubios HRV Premium 3.0.2 (Tarvainen et al., 2014), providing the calculation of time- and frequency-domain indices of vagally-mediated HRV (vmHRV), i.e., the root mean square of successive differences (RMSSD-HRV) and the high-frequency band (HF-HRV, 0.15-0.4 Hz), respectively (Laborde et al., 2017), which are highly correlated (Kleiger et al., 2005). Artifact correction was conducted manually in Kubios, if necessary. To compare across task conditions, HRV was calculated for both inclusion (50 sec) and exclusion (110 sec) conditions.

2.5.2. Neuroendocrine responses

Neuroendocrine assessments were conducted after midday, as the afternoon has been suggested as favorable for studying the provoked stress response of neuroendocrine hormones (King and Liberzon, 2009) (for more details see Methods S1). Blood samples were taken from an i.v. catheter, with the exception of two participants in the NMPOU group because of needle phobia and problems with placing the i.v. catheter. In total, five blood samples per participant were collected about 20 minutes before (baseline, T1) and +10 (T2), +20 (T3), +30 (T4), and +60 (T5) minutes after psychosocial stress (Kirschbaum et al., 1999) induced by the Cyberball task using BD Vacutainer® containing Lithium Heparin. Plasma samples were stored at -80°C until analysis. After completion of the study, all samples were analyzed via immunoassays in a specialized laboratory at the Technical University of Dresden (Dresden LabService) to assess neuroendocrine stress parameters of the HPA axis, i.e., adrenocorticotropic hormone (ACTH) and cortisol. ACTH data of one participant in the

control group were not detectable due to hemolytic plasma samples. Single time-point missing data in plasma cortisol and ACTH were estimated by calculating the mean of the flanked plasma values, if the missed time-point occurred between two successfully acquired samples. If missing values occurred for the first or last sample, the average difference of the respective group between the first and the second or the fourth and fifth value was subtracted from the second or fourth value of the individual, respectively.

2.6. Statistical Analyses

Statistical analyses were conducted using SPSS 23.0 software. Frequency data were analyzed by means of Pearson's χ^2. Quantitative between-group data were either analyzed by independent t-tests or Mann-Whitney-U-tests, if non-normally distributed. In order to analyze changes within groups, paired samples t-tests or, in the case of non-normal distribution, Wilcoxon signed rank tests were applied. Additional analyses of covariance (ANCOVA) for all subjective responses to social rejection and the social network size were conducted to control for age and sex distribution because of reported associations with prosocial behavior (Beadle et al., 2015; Kret and De Gelder, 2012; Miller et al., 1991; Smith et al., 2015).

Changes in the distribution of cortisol and ACTH over time were analyzed using repeated measures ANOVA with *time* (5 time points) as within-subjects factor and *group* as between-subjects factor. Greenhouse-Geisser correction was used, if the assumption of sphericity was violated. Furthermore, area under the curve with respect to increase (AUC_i) was calculated for the hormone profiles based on Pruessner et al. (2003). Furthermore, to control for baseline (T1) hormonal levels and body mass index (BMI), ANCOVAs with the respective covariates were conducted with the neuroendocrine data and HRV, respectively (Koenig et al., 2014). Log-transformation was applied when data showed right-skewed distribution (log_{10}). Pearson's product-moment correlations within the NMPOU group were used to investigate associations between drug use severity and physiological stress responses. The confirmatory statistical comparisons were carried out with a significance level of $p<0.05$ (two-tailed) with the exception of the correlation analyses, where $p<0.01$ (one-tailed) was applied in order to

avoid alpha-error accumulation. Cohen's *d* effect size (Cohen, 1988) was calculated by the means and pooled standard deviations of both groups.

Based on the recent study by Garland et al. (2017) investigating changes in HRV between opioid-misusers and non-misusers in chronic pain patients, we calculated an a priori power analysis using G*power 3.1 (Faul et al., 2009) assuming an effect size of $\eta^2_{partial}$=.07, α-error probability of 5%, power of 90%, and a correlation of 0.5 among repeated measures for the 2 x 2 mixed ANOVA within-between interaction, which suggested a total minimum sample size of N=38.

Considering the different analgesic potentials of different opioids, morphine equivalents (ME) for each opioid (self-report and hair concentration) were calculated based on ME conversion factors per mg of opioid, as recently reported (Kroll et al., resubmitted).

3. RESULTS

3.1. Demographic characteristics and drug use

As previously reported, controls and individuals with NMPOU did not significantly differ in demographic variables (Table 1) (Kroll et al., resubmitted). However, individuals with NMPOU showed a higher BDI score, in line with prior reports of opioid users (Ersche et al., 2006). Individuals with NMPOU mostly reported only mild opioid craving at a median of 3.00 (range 1-8) with absence of severe withdrawal symptoms before and after the Cyberball paradigm (Table 1). In the NMPOU group, the most frequent used single opioid was dihydrocodeine (43.5%), followed by codeine, morphine, and buprenorphine (each 8.7%). Three subjects (13%) reported mixed use of different opioids (Table S1). Severe opioid use disorder according to DSM-5 within the NMPOU group was diagnosed for 56.5% (n=13) and drug reports as well as hair samples revealed a clear dominance of opioid use compared to other substance classes (Table 1). One subject from the NMPOU group showed insufficient availability of hair samples to detect opioid metabolites; however, the urine toxicology was positive for opioids, therefore we decided to include the participant in the analyses. All other 22 individuals with NMPOU showed considerable opioid concentrations in hair samples, whereas hair concentrations of any psychotropic drugs in the control group were below established cut-off values (Cooper et al., 2012) except for one participant showing very low MDMA concentrations, which were considered as negligible. Twelve urine samples within the NMPOU group tested positive for opioids. However, we decided to include them in the analysis and to consider potential acute effects in separate analyses.

3.2. Physiological stress response

3.2.1. Heart rate variability

As expected, RMSSD-HRV and HF-HRV were highly correlated in the inclusion (r=.88, p<.001) and exclusion condition (r=.92, p<.001). Therefore, only RMSSD-HRV results are reported in the following, whereas HF-HRV results are additionally shown in the tables. No significant group differences for RMSSD-HRV were found between individuals with NMPOU and controls, as shown in Table 2, even

after controlling for BMI (*p*'s>.05). However, paired-samples t-tests for controls revealed a significant increase of RMSSD-HRV from the inclusion to the exclusion condition (t(28)=-2.42, *p*<.05), which was also seen at a trend level in the NMPOU group (t(22)=-1.99, *p*=.056). Of note, correlation analyses showed negative correlations between opioid craving and HRV variables in the NMPOU group (r=-.49, *p*<.01, Table S2a). Due to previously reported associations between craving and HRV in alcohol-dependent patients (Ingjaldsson et al., 2003; Quintana et al., 2013), we divided the NMPOU group using a median split of the craving NRS ratings into "no/low craver" (LowC, n=15) and "medium/high craver" (HiC, n=8). Demographic characteristics and drug use are shown in Table S3. Based on the results of Ingjaldsson et al. (2003) and Quintana et al. (2013), we assumed that higher opioid craving would be accompanied by lower HRV. Therefore, one-tailed statistical analyses were carried out for HRV. ANOVAs revealed statistical significance for RMSSD-HRV inclusion (F(2,49)=3.89, *p*<.05) and for RMSSD-HRV exclusion (F(2,49)=5.33, *p*<.01). Specifically, HiC showed lower RMSSD-HRV, compared to controls (*d*=.84) and LowC (*d*=1.15), whereas controls and LowC showed no differences in HRV (*d*=.31, Table S4). Paired-samples t-tests revealed a significant increase in RMSSD-HRV from inclusion to exclusion only for controls and LowC (t(14)=-2.01, *p*<.05) but not for HiC (t(7)=-.43, *p*=.341), as shown in Fig. 1.

Because low HRV is also associated with depression (Beauchaine and Thayer, 2015) and individuals with NMPOU reported higher BDI scores than controls, we additionally divided the NMPOU group using a median-split (median BDI=8) into high (n=11) and low BDI scored subjects (n=12). However, independent t-tests for RMSSD-HRV revealed no differences between both BDI groups in either the inclusion or the exclusion condition of the Cyberball task (Fig. S1). Individuals with low BDI scores showed a significant increase of RMSSD-HRV during exclusion (t(10)=-1.97, *p*<.05, one-tailed), which was similarly seen at a trend level for individuals with high BDI scores (t(11)=-1.48, *p*=.084, one-tailed). Finally, correlation analyses between BDI sum score and electrophysiological parameters, as well as craving, showed no significant associations (Table S2b).

3.2.2. Spontaneous fluctuation in the skin conductance

T-test analyses revealed no significant group differences in SF over the whole task or in the exclusion condition alone (Table 2). Additional ANOVAs with craving group as fixed factor and SF variables as dependent factors again revealed no group differences in SF in skin conductance (Table S4), indicating similar response of the SNS in all groups.

3.2.3. Cortisol and ACTH

The repeated measures ANOVA for ACTH revealed no significant *group x time* interaction ($F(2.2,100.9)=1.78$, $p=.171$) and no *group* effect ($F(1,47)=1.47$, $p=.232$). Similar results were found for cortisol (*group x time* interaction: $F(1.9,92.9)=1.92$, $p=.154$; *group* effect: $F(1,48)=1.04$, $p=.313$). However, the NMPOU group alone showed an increase of ACTH and cortisol after social exclusion (Fig. 2), which was supported by significant group differences in ACTH and cortisol peaks corrected for baseline (T1) about 30 minutes after the Cyberball (Fig. 2) and by a trend for the AUC_i cortisol ($t(48)=-1.73$, $p=.089$). Similar results were found for craving groups showing medium effects sizes for the AUC_i between controls and craving groups but not between LowC and HiC (Table S4).
As elevated cortisol and hyperactivity of the HPA axis have been commonly found in depressed patients (Herbert, 2013), we additionally conducted repeated measures ANOVAs (2 groups x 5 time points) comparing NMPOU individuals with high and low BDI scores. No significant differences in ACTH and cortisol levels were found between high and low BDI groups (ACTH: $F(1,19)=.68$, $p=.419$; cortisol: $F(1,19)=1.85$, $p=.190$), although NMPOU individuals with high BDI scores showed slightly lower hormone levels across all time points (Fig. S2). Moreover, no significant *group x time* interactions were found (ACTH: $F(1.9,36.2)=.65$, $p=.523$; cortisol: $F(2,37.4)=.16$, $p=.847$).

3.3. Self-rating scales

3.3.1. PANAS and Cyberball questionnaire

Group differences were found for PA prior to the Cyberball (Table 2) indicating less positive mood in the opioid users. However, comparisons of PA and NA before and after the Cyberball task revealed

decreased positive and increased negative state only for the controls (PA: t(28)=2.227, p<.05; NA: Z= -2.088, p<.05) but not for the NMPOU group (PA: t(22)=.478, p=.637; NA: Z=- .317, p=.751). No group difference was found in the estimation of percentage of received balls during the game and in feelings of being excluded or included (p's>.05). These results did not change even after correction for age and sex. HiC revealed stronger feelings of exclusion compared to LowC and controls. However, mood changes were only found in controls (Fig. 4), whereas LowC reported a slight decrease of NA after the Cyberball game (Table S4). Correlation analyses with feelings of inclusion/rejection revealed no significant association (p<.001) with physiological stress responses over all groups (Table S6).

3.3.2. Social network size

The independent t-test revealed a significantly smaller social network size (t (50)=2.39, p<.05) for individuals with NMPOU compared to controls (Table 2). The group effect remained significant even after correction for sex, age, and employment status (F(1,47)=6.22, p<.05), which we included as a covariate as unemployment might have an impact on social network size (Kroll et al., 2018). The ANOVA for craving groups revealed significance with a clear linear trend (p<.01), significant Sidak post hoc tests for controls and HiC (p<.05), and strong effects sizes (Table S4) suggesting a smaller social network specifically in HiC. Finally, social network size was not correlated with depressive symptoms measured with the BDI (Table S2b).

3.4. Opioid use parameters: Morphine equivalents hair concentration and acute opioid effects

Log-transformed ME hair concentration was not correlated with any stress response parameters of the ANS within the NMPOU group (p's >.05). However, AUC_i ACTH was positively correlated with ME hair concentrations (r=.53, p<.01, Fig. 3), which was also detected in AUC_i cortisol (r=.40, p<.05), but with a smaller effect. Self-rating data revealed no correlation with ME hair concentration. In order to assess potential acute and postacute drug effects, we compared social stress parameters between individuals with positive and negative urine screenings for opioids (Table S5). Although no significant

differences in social stress parameters were found between groups, effect sizes indicated slightly lower ratings for feelings of inclusion (*d*=.80) and higher ratings of negative mood pre- (*d*=.68) and post-Cyberball (*d*=.61) for individuals with positive opioid urine screenings. However, paired-samples t-tests revealed no changes in mood before and after the Cyberball as well as in HRV parameters during social exclusion for both groups (*p*'s>.05).

4. DISCUSSION

The aim of our study was to investigate potential effects of chronic NMPOU on subjective and physiological stress responses to social rejection. We found that high opioid cravers (HiC) specifically showed difficulties in emotion regulation during social rejection, whereas controls and low cravers (LowC) displayed a better psychophysiological adaptation to psychosocial stress and emotion regulation indexed by HRV. Furthermore, no group differences were observed for SF in skin conductance, indicating preserved sympathetic activity in individuals with NMPOU. Interestingly, we found a pronounced increase of ACTH and cortisol for the NMPOU group compared to controls after the Cyberball, suggesting a hyperreactivity of the HPA axis to social rejection, which was related to the opioid hair concentration in a dose-dependent manner. Furthermore, self-ratings indicated that individuals with NMPOU were aware of being excluded but less subjectively affected by social rejection than controls. Finally, NMPOU individuals exhibited smaller social network size.

The main finding of our study was that individuals with NMPOU showed a dysregulated stress response to social exclusion, specifically in the activity of the PNS and HPA axis. We found difficulties in emotion regulation during social exclusion for HiC, indexed by lower and unchanged HRV during the Cyberball task. In contrast, LowC and controls displayed increased HRV during social exclusion, suggesting adequate adaptation to social rejection. Our results are consistent with the findings by Ingjaldsson et al. (2003), who reported lower HRV for high craving alcoholics compared to controls. Low HRV has been associated with several psychopathological disorders such as depression, anxiety disorder, posttraumatic stress disorder, and alcohol dependence (Beauchaine and Thayer, 2015;

Quintana et al., 2013). Furthermore, high HRV is associated with better and more successful emotion regulation of negative affect, whereas lower resting HRV was shown in individuals with difficulties in emotion regulation (Williams et al., 2015). As stated by the *Neurovisceral Integration Model* (NIM) of Thayer and Lane (2009), the heart is under the tonic inhibitory control of the PNS over the SNS, as indexed by HRV. Neuro-structural correlates of this process are represented by the inhibitory control of subcortical structures, such as the amygdala, via the PFC and its interconnections with the ACC and Insula (Thayer et al., 2012). These brain areas are also associated with emotion processes and social pain as well as with high opioid receptor density (Eisenberger, 2012; Nummenmaa and Tuominen, 2017). Furthermore, alterations of structural and functional connectivity of the amygdala, ACC, insula, and PFC have been found in opioid-dependent patients (Upadhyay et al., 2010; Younger et al., 2011). Consistent with these findings, the present results support the NIM and indicate that chronic NMPOU might lead to difficulties in emotion regulation and adaptation to social rejection, with a specific modulation by craving. This interpretation is also consistent with a recent study reporting attenuated HRV response during negative emotion regulation in chronic pain patients misusing prescription opioids (Garland et al., 2017). Participants were required to reappraise images with negative content as well as to savor and subjectively experience images with positive content. Interestingly, chronic pain patients misusing opioids showed no change in HRV from baseline to the reinterpretation of negative emotions, whereas non-misusers revealed an increase of HRV (Garland et al., 2017). Furthermore, prescription opioid misusers showed significantly higher craving scores than non-misusers and morphine equivalent daily opioid dose (MEDD) was not correlated with HRV, which is in line with the results of our study. In contrast to our findings regarding the PNS, our SCL measurements indicated similarities in sympathetic responses between individuals with NMPOU and controls during social exclusion. This is coherent with a recent study investigating SCR to emotion-eliciting videos in opioid-substituted patients, which reported no group effects, compared to controls, for the SCR (Biernacki et al., 2018). Taken together, the present results showing alterations in the PNS in opioid users, indexed by the HRV, but no changes in the SNS, indexed by SF in skin conductance, indicates that chronic opioid use might lead to an imbalance within the ANS.

Analogously, chronic opioid use is commonly associated with dysfunction of the HPA axis, as reflected by suppressed glucocorticoid levels after acute opioid administration in animals and humans, as well as by elevated cortisol levels during opioid withdrawal symptoms in heroin users (Kreek et al., 2012; Vuong et al., 2010; Walter et al., 2013). Our finding of equal ACTH and cortisol baseline levels across groups leads to the conclusion that strong withdrawal effects can be excluded as a potential confounding factor in our study. In contrast to the findings by Bershad et al. (2015), reporting attenuated cortisol response to psychosocial stress after administration of buprenorphine, our results indicated increased activity of the HPA axis only for the NMPOU group after the Cyberball task, whereas controls revealed no stress response to social exclusion. However, Bershad et al. (2015) investigated acute effects of a partial MOR agonist, whereas our study examined chronic effects of, primarily, full MOR agonists. Furthermore, social stress was induced by using the TSST, which might activate different biological stress response processes to the psychosocial stress induced by social exclusion, as implemented in our study. This is supported by the theory that cortisol release is caused by stressors signaling the need for power (i.e., mobilization of stored energy), rather than the need for affiliation (Weik et al., 2017). Accordingly, some previous studies using the Cyberball paradigm in healthy participants were not able to find stress-induced cortisol increase after social exclusion (Bass et al., 2014; Jobst et al., 2015; Radke et al., 2018; Zwolinski, 2012). Therefore, non-response of the HPA axis in the control group of our study might be explained by this theory. Individuals with NMPOU, however, showed an increase of ACTH and cortisol, indicating a hyperreactivity of the HPA stress axis to social exclusion in chronic opioid users, which was also dose-dependent. Therefore, our results support the notion of an opioid-induced dysfunctional HPA axis, albeit in a different direction to that reported previously after acute opioid administration. Given that animal studies have reported inhibited production of endorphins and down-regulation of MOR after chronic opioid administration (Sprouse-Blum et al., 2010) and that endorphins were postulated to inhibit and counteract over-activation of the HPA axis in response to stress (Bali et al., 2015), our findings might be explained in terms of: 1.) Chronic opioid use leads to down-regulation of endogenous opioid release, resulting in hypersensitivity to social stress, which might also result from

dysfunctional emotion regulation, and therefore encourages maintenance of opioid use and opioid relapse. 2.) A lower baseline endorphin level entailing hypersensitivity to social stress is a predisposing factor in individuals who are prone to use opioids to reduce social distress. This second view is supported by the fact that anxiety disorders including social phobia are more frequent in individuals with NMPOU (Becker et al., 2008). Moreover, existing preclinical and human evidence for the antidepressant-like effects of opioids highlights their potential for self-medication in individuals with NMPOU (Martins et al., 2012; Pecina et al., 2018). However, chronic use of other, non-opioid, substances is also highly associated with mood and anxiety disorders, suggesting a potential self-medicating strategy not only for individuals with NMPOU but for substance use disorders in general (Turner et al., 2018). Further longitudinal studies should target and address these different causal explanations.

In addition to the finding that plasma cortisol levels at baseline (T1) did not statistically differ between individuals with NMPOU and controls, cortisol hair concentrations also revealed no differences between groups. Given that hair cortisol can be interpreted as a biological marker of chronic stress (Russell et al., 2012), present results thus suggest no elevated stress exposure in the NMPOU group over the last months. However, this could also be a result of the chronic opioid use, potentially instrumentalized for its stress-reducing effects in this population (Muller and Schumann, 2011).

Self-ratings revealed negative changes in mood after social exclusion only for controls, which is an indicator of the task's validity and supports the theory that psychosocial stress induced by social exclusion might activate different mechanisms of stress response than stress-induction by the TSST. Although individuals with NMPOU showed a hyperreactivity of the HPA axis to social exclusion, no changes in mood were found after the Cyberball task. Furthermore, no group differences in the estimation of the number of received balls or in feelings of exclusion were found, which is in contrast to results examining acute opioid effects using the Cyberball (Bershad et al., 2016). Therefore, our findings suggest that individuals with chronic NMPOU were aware of being excluded, but nevertheless felt less affected emotionally by social rejection. Interestingly, HiC revealed stronger

feelings of being excluded compared to LowC and controls, which might result from a more pronounced dysfunctional emotion regulation in the most severe cases of NMPOU. The contrary findings to those of Bershad et al. (2016) might relate to the fact that they investigated acute opioid effects and used a partial MOR agonist, whereas participants in our NMPOU group were asked to abstain from opioids on the testing day and showed chronic NMPOU of, primarily, full MOR agonists. Nevertheless, some urine samples from the NMPOU group did test positive for opioids. Additional statistical analyses revealed no differences in subjective and physiological stress responses between individuals with NMPOU showing positive or negative urine toxicology for opioids. Given that the mean abstinence time for individuals testing positive for opioids was 14.3 hours (range 1-27 hours) and that the Cyberball task began at about 2.5 hours after measurement onset for the NMPOU group, positive urine toxicology might not represent acute drug effects in our NMPOU sample.

Assessments of real-life social functioning by the SNQ revealed a smaller social network size in individuals with NMPOU. Therefore, our findings support the BOTSA suggestion that exogenous opioids might replace the need for social contacts and decrease affiliative behavior, resulting in a smaller social network size and in being less emotionally affected by social rejection. Similarly, the study by Johnson and Dunbar (2016) indicated an association between the endogenous μ-opioid system and social network sizes. However, a smaller network size was also reported in cocaine and stimulant polysubstance users (Kroll et al., 2018; Preller et al., 2014). This raises the question of whether this phenomenon is caused by a predisposing factor that might also facilitate substance use, or whether chronic opioid use leads to a saturation of the MOR and therefore decreased affiliative behavior resulting in fewer social contacts. However, several studies in animals and some in humans have reported alterations in the endogenous opioid system after chronic cocaine administration, indicated by an increase in MOR (Kreek et al., 2012). Therefore, the findings of a smaller network size in both cocaine and opioid users might support the assumption that the endogenous opioid system plays a crucial general role in affiliative behavior and also provide evidence supporting the BOTSA in humans. Longitudinal studies should further test and clarify this question.

Our study has some limitations that should be noted: First, the sample size of the NMPOU group was small because of our strict exclusion criteria and limited prevalence of NMPOU in Switzerland. However, as a positive feature, our NMPOU group consisted of relatively pure opioid users, objectively confirmed by urine and hair analyses. Furthermore, required effect sizes were calculated in advance using Cohen's *d* to address this limitation. Second, due to our study design, we were not able to compare self-ratings, SCR, and neuroendocrine responses between the inclusion and exclusion conditions of the Cyberball task. Therefore, we cannot unequivocally ensure that our results were distinct stress-related responses to social exclusion. However, affect changes after the Cyberball in the control group indicate that the task was valid. Third, cross-sectional designs do not allow for straightforward interpretations regarding causality. Although our sample displayed relatively pure opioid use and lacked confounding factors such as pain disorders or psychiatric axis-I disorders, future longitudinal studies might elucidate the relationship between chronic prescription opioid use and the response to social rejection more clearly.

To the best of our knowledge, this is the first study investigating effects of chronic opioid use on subjective and physiological stress responses to a psychosocial stressor such as social rejection. With respect to the opioid crisis in the U.S. and also increased NMPOU in Europe, it is important to understand the underlying mechanisms as well as the long-term sequelae of chronic opioid use. Our findings indicate that chronic NMPOU is not only accompanied by an abnormal functioning of the HPA axis but also by a dysregulation of the PNS. Individuals with high opioid craving revealed pronounced difficulties in emotion regulation and adaptation during social rejection, as indexed by HRV. Given that HRV is not only a downstream measurement but that HRV itself can affect emotion regulation and associated brain networks, present findings might contribute to guiding the development and implementation of new treatments of opioid dependence, such as HRV biofeedback, to restore ANS balance (Mather and Thayer, 2018). This notion is supported by previous results showing HRV biofeedback to be efficient in the treatment of heroin use with depressive symptoms and also of drug craving (Eddie et al., 2014; Lin et al., 2016). Although subjective self-ratings in the NMPOU group implied that their perception of social exclusion was preserved, chronic

opioid users were less subjectively affected by social rejection and reported a smaller social network size than controls, which is consistent with the BOTSA and previous animal studies (Machin and Dunbar, 2011). Given that functional social support has been found to prevent drug relapse and that inadequate stress response and craving are crucial risk factors for drug relapse, our results have important implications for future opioid dependence interventions targeting these deficits.

Contributors

SLK and BBQ designed the study. BBQ provided the funding for the study. SLK and MT conducted the assessments. JFT and DW supported the HRV analyses and data interpretation. MS supported the SCL analyses. CK conducted the plasma analyses. MRB and TMB conducted the hair analyses. SLK conducted data preprocessing and statistical analyses. SLK and BBQ wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Declarations of interest

Funding

The study was funded by the Herzog-Egli Foundation and starting grants of the Medical Faculty of the University of Zurich and of the Psychiatric Hospital of the University of Zurich to BBQ. SLK was financed by a grant of the Swiss National Science Foundation (105319_162639/1) to BBQ.

Conflict of interest

None.

Acknowledgements

We are grateful to Matthias Humm (Drug Information Centre, DIZ, and www.saferparty.ch), Markus Herdener (University Hospital for Psychiatry Zurich and Centre for Use Disorders, ZAE), and Andreas Wahl-Kordon (Oberbergklinik Schwarzwald) for supporting the recruitment. Moreover, we thank Emilija Nikolic, Franziska Bieri, Jael Bernath, Kateryna Mudrak, and Claudia Hänggi for their excellent support with the assessment of the participants. Finally, we thank all members of our research group for their time to pretend playing the Cyberball with the participant in our study. Special thanks go to

David Cole for commenting and proofreading the manuscript and to Hannes Sigrist for his support with dry ice and for helping us with the transportation of the plasma samples.

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Fig. 1. Average root mean square successive difference (RMSSD) in heart rate during inclusion and exclusion condition of the Cyberball task.

Paired-samples t-tests with $p<.05$*. Error bars reflected ±1 standard error.

CB: Cyberball, HiC: high craver, LowC: low craver.

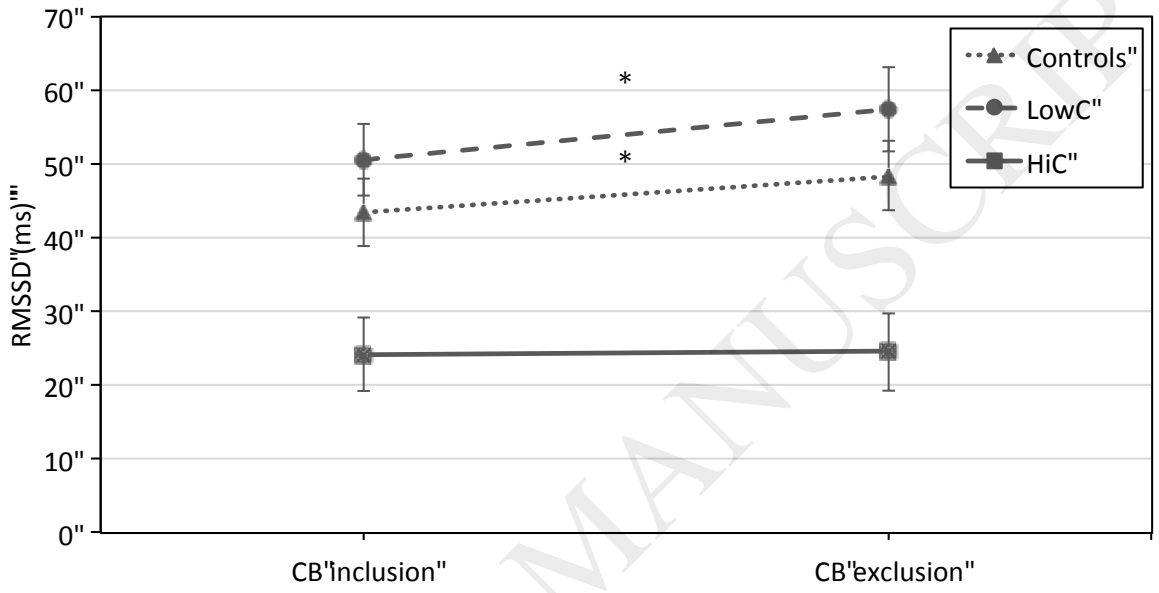

Fig. 2. Mean ACTH (above) and cortisol (below) plasma concentrations before and after the Cyberball task.

Error bars reflected ±1 standard error. Shaded area highlights the duration of the Cyberball task.

ANCOVAs for plasma samples corrected for baseline (T1) are indicated by p<.05*.

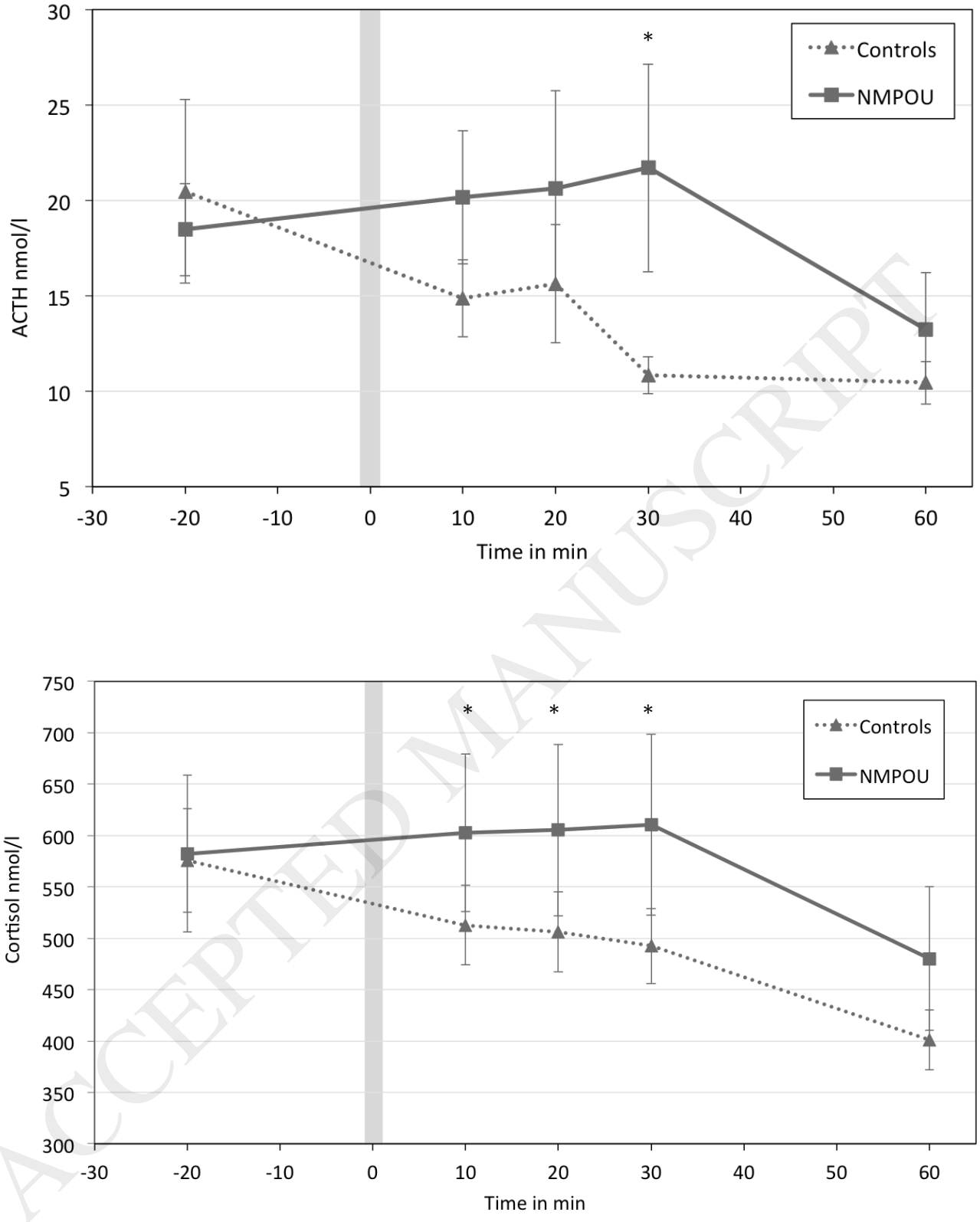

Fig. 3. Scatter plot of the area under the curve to increase (AUC_i) for ACTH and cumulated morphine equivalents (ME) of opioid hair concentration. Pearson correlation analysis showed significant dose-dependent effects of opioids on ACTH AUC_i with $p<.01$.

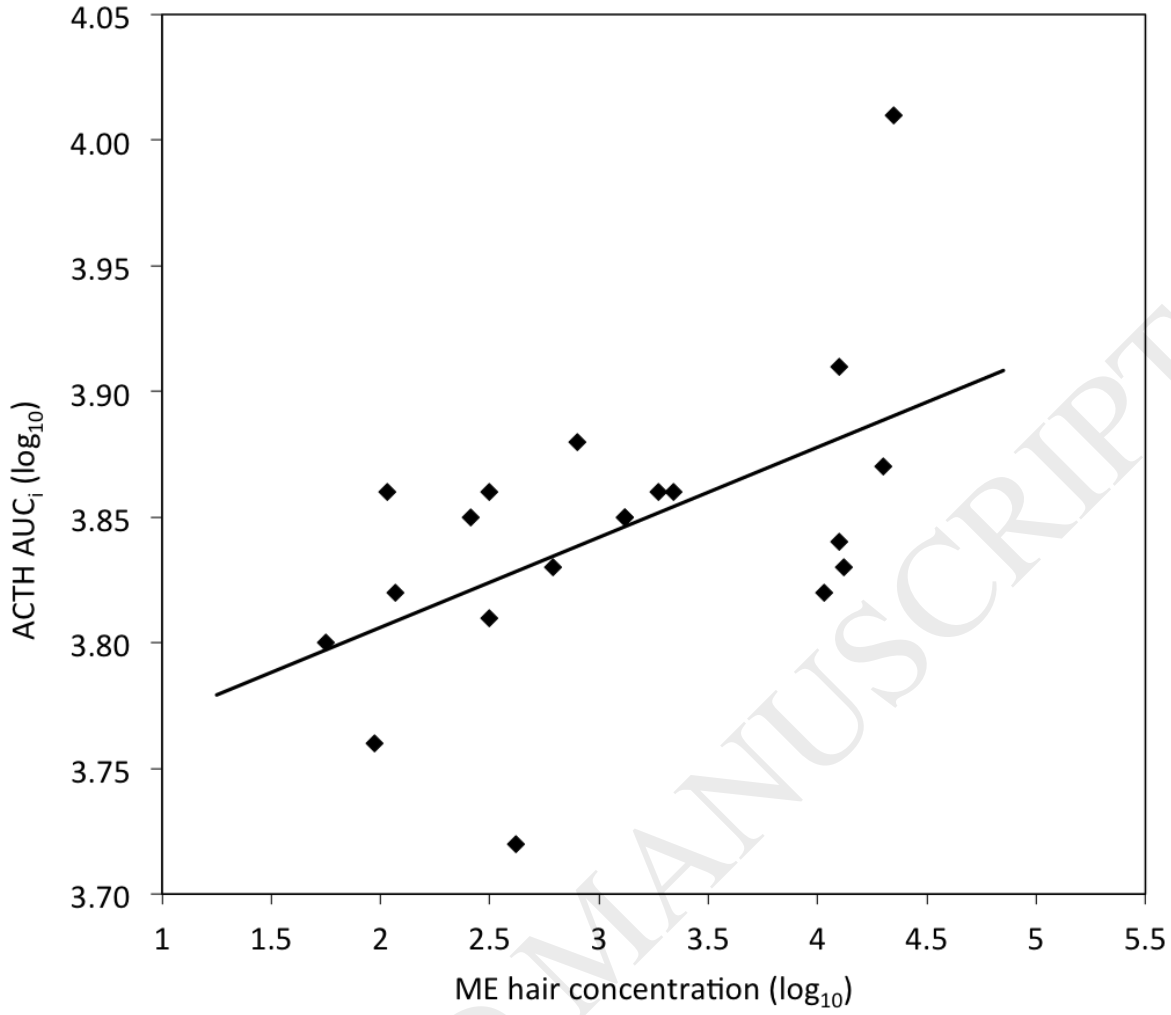

Fig. 4 Mean Positive and Negative Affect Schedule (PANAS) scores before and after the Cyberball task.

Paired samples t-test for PA (two-tailed) and Wilcoxon Signed-Rank test for NA p<.05*. Error bars reflected ±1 standard deviation. CB: Cyberball, NA: Negative affect, PA: Positive affect.

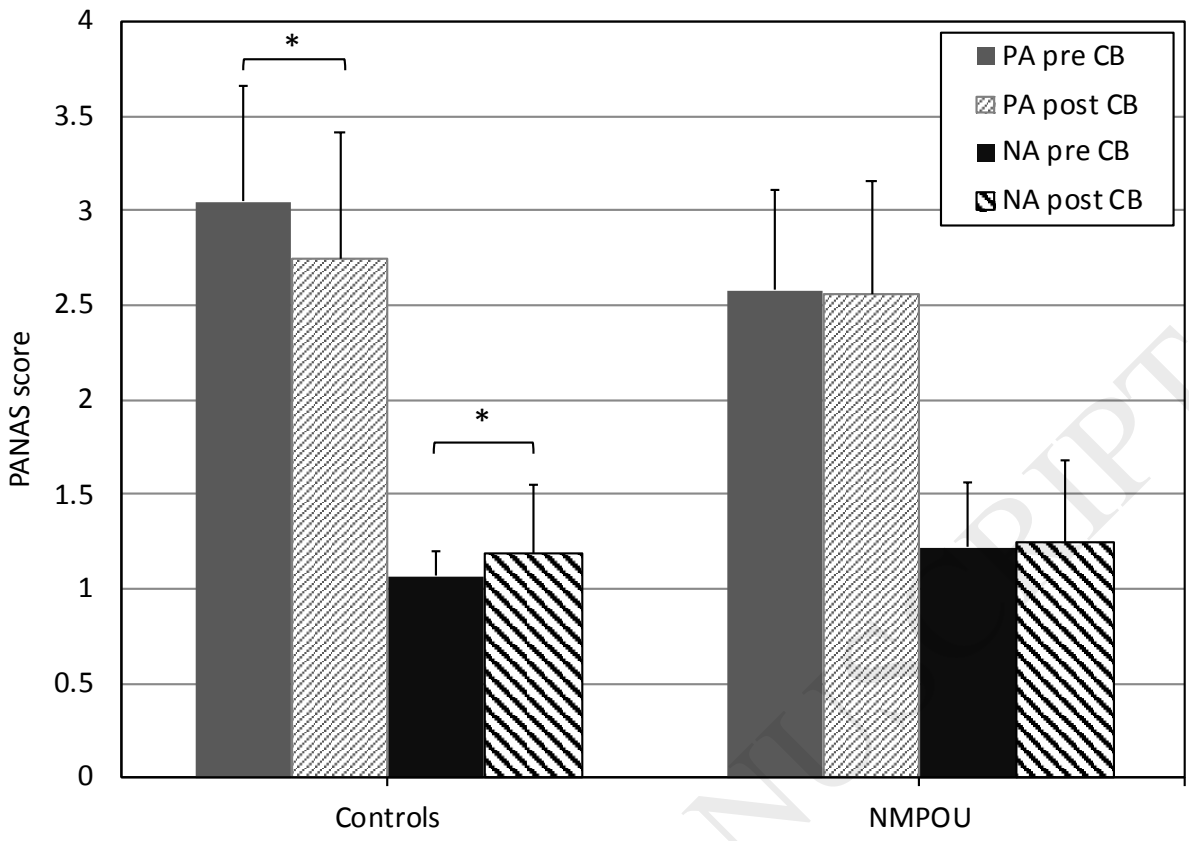

Table 1 Demographic data and drug use (means and standard deviations)

	Controls (n=29)	**NMPOU** (n=23)	**Value**	***df***	***p***
Female/male	10/19	6/17	$\chi^2 = 0.42$	1	0.515
Age	26.55 (8.1)	27.96 (10.3)	t = -0.55	50	0.582
Body mass index (BMI)	22.45 (2.6)	23.68 (3.6)	t = -1.42	50	0.162
Years of education	11.48 (1.5)	11.17 (2.0)	t = 0.62	39.20	0.542
Verbal IQ	105.24 (11.3)	106.39 (11.2)	t = -0.37	50	0.717
Employment (y/n)	28/1	19/4	$\chi^2 = 2.87$	1	0.090
BDI sum score	3.00 (3.3)	9.39 (7.7)	t = -3.71	28.35	**<0.001**
Cortisol hair concentration pg/mg	11.05 (12.5)	17.50 (20.6)	t = -1.82	50	0.075
Smoker/non-smoker	18/11	17/6	$\chi^2 = 0.82$	1	0.366
Cigarettes per week[a]	45.42 (35.7)	81.76 (57.9)	t = -2.20	26.35	**0.035**
Fagerström test (FTND)[a]	1.28 (1.6)	2.59 (2.3)	t = -1.92	28.06	0.065
Alcohol gram/week	69.65 (65.5)	51.28 (62.2)	t = 1.03	50	0.309
Opiates					
Times per week	-	3.88 (3.0)			
ME mg/week	-	543.35 (964.9)			
Years of use[b]	-	2.88 (0.5 - 28.0)			
Craving (NRS)	-	3.35 (2.7)			
OOWS pre Cyberball[b]	-	0.00 (0-1)			
OOWS post Cyberball[b]	-	0.00 (0-4)			
Positive urine tests (y/n)	0/29	12/11			
ME hair concentration pg/mg	0.82 (4.2)	4 703.46 (7 065.6)			
Cannabis					
Grams per week	0.07 (0.3)	0.36 (0.6)	U = 191.0		**0.023**
Years of use	3.73 (4.2)	4.78 (4.8)	U = 300.5		0.540
Positive urine tests (y/n)	0/29	5/18			
Amphetamine					
Lifetime gram[b]	0.00 (0.0 - 1.3)	0.00 (0.0 - 5018.5)			
Positive urine tests (y/n)	0/29	0/23			
Hair concentration pg/mg	0.00 (0.0)	19.57 (83.6)	U = 304.5		0.120
MDMA					
Lifetime gram[b]	0.00 (0.0 - 0.6)	0.10 (0.0 - 65.2)			
Positive urine tests (y/n)	0/29	0/23			
Hair concentration pg/mg	13.62 (59.9)	265.65 (505.3)	U = 228.0		**0.004**
Cocaine					
Lifetime gram[b]	0.00 (0.0 - 1.8)	0.10 (0.0 - 298.4)			
Positive urine tests (y/n)	0/29	1/22			
Hair concentration pg/mg	1.55 (8.4)	292.17 (557.3)	U = 195.0		**<0.001**

Significant *p*-values ($p<.05$) are shown in bold. T-test and Chi^2 for frequency distribution two-tailed.
[a] Only within smokers
[b] Median (range) reported
BDI: Beck's Depression Inventory, FTND: Fagerström test of nicotine dependence, ME: morphine equivalent, NRS: numeric rating scale (1-10), OOWS: objective opioid withdrawal scale (0-12).

Table 2: Independent t-tests of dependent variables (means and standard deviations)

			Controls (n= 29)	NMPOU (n= 23)	t	*df*	*p*	Cohen's *d*
Physiological responses to social rejection								
HRV								
	RMSSD-HRV inclusion	*	43.36 (24.5)	41.34 (21.3)	0.31	50	0.756	0.09
	RMSSD-HRV exclusion	*	48.42 (25.2)	45.98 (25.4)	0.35	50	0.731	0.10
	log HF-HRV inclusion		6.34 (1.3)	6.26 (1.3)	0.23	50	0.820	0.06
	log HF-HRV exclusion		6.59 (1.1)	6.30 (1.3)	0.88	50	0.385	0.25
SF in skin conductance								
	SCL (µS)		3.02 (1.9)	2.91 (1.5)	0.23	50	0.818	0.07
	SCL exclusion (µS)		2.98 (1.8)	2.88 (1.5)	0.20	50	0.842	0.06
	DCM		0.12 (0.1)	0.12 (0.1)	0.01	50	0.995	0.00
	DCM exclusion		0.07 (0.1)	0.06 (0.1)	0.55	50	0.587	0.15
Self-ratings								
PANAS								
	PA pre CB	*	3.05 (0.6)	2.58 (0.5)	2.93	50	**0.005**	0.76
	PA post CB	*	2.74 (0.7)	2.56 (0.6)	1.05	50	0.298	0.29
	NA pre CB[a]	*	1.07 (0.1)	1.22 (0.3)	-1.82		0.068	0.60
	NA post CB[a]	*	1.19 (0.4)	1.25 (0.4)	-0.37		0.710	0.15
CB questionnaire								
	Feelings of exclusion		6.62 (1.5)	6.30 (2.2)	0.62	50	0.539	0.17
	Feelings of inclusion		3.45 (1.4)	2.83 (1.5)	1.56	50	0.126	0.43
	Estimated % balls received		16.28 (7.7)	16.65 (9.7)	-0.16	50	0.876	0.04
	Difficulty of believing the task		2.52 (1.3)	1.91 (1.2)	1.70	50	0.095	0.47
SNQ								
	Social network size total		21.31 (8.0)	15.83 (8.5)	2.39	50	**0.020**	0.64

Significant *p*-values (*p*<.05) are shown in bold.
Paired samples t-test (two-tailed) or Wilcoxon Signed-Rank test *p*<.05*
[a] Mann-Whitney U tests
CB: Cyberball, DCM: dynamic causal modeling, HF: high frequency, HRV: heart rate variability, NA: negative affect, PA: positive affect, RMSSD: root mean square of successive differences, SCL: skin conductance level, SF: spontaneous fluctuation